PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/050899	01.03.2005	02.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N9/10, C12N15/62, G01N33/535

Applicant
EPFL ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Barnas, C

Telephone No. +49 89 2399-7469

ATTACHMENT E

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/050899

10/591159

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-8

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 1-8

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	14-30, 32-36
	No:	Claims	9-13, 31, 37
Inventive step (IS)	Yes:	Claims	
	No:	Claims	14-30, 32-36
Industrial applicability (IA)	Yes:	Claims	1-37
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

The documents mentioned in the present Communication are numbered as in the Search Report. D1 corresponds to the first document of the Search Report, D2 to the second document etc.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The examination has been carried out for the subject matter that has been searched, i.e. mutant alkyltransferases with mutations as described in 9-30 and subject matter connected therewith as described in claims 31-37.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Art. 33(2) PCT, Novelty

D1 discloses an human alkyltransferase (hAGT) mutant that contains (I) two mutations already disclosed in D2 that increased the activity and (ii) three additional mutations (Lys125Ala, Ala127Thr, Arg128Ala, disclosed in D1) that disrupt the DNA binding of hAGT. Thus, the two mutations from D2 were Asn157Gly, Ser159Glu (PGEG, see Table 1 in D2).

D1 discloses therefore the mutant Lys125Ala, Ala127Thr, Arg128Ala, Asn157Gly, Ser159Glu.

D1 is, therefore novelty destroying for **claims 9-13.** D1 further anticipates the novelty of the subject matter of **claims 31 and 37**.

2. Art. 33(3) PCT, Inventive Step

2.1. In order to obtain an hAGT mutant with increased activity and disrupted DNA binding, the skilled person would also combine the three mutations of **D1** listed above with further mutations that confer increased activity as disclosed in **D2** (MWSV, PGEA, PGNW,

PPQC, PGQW, PGSG, see Table 1) using routine experimentations, without the exercise of inventive skill. Mutations as combined in **claim 9 (f) and 10(f)** are, therefore not inventive.

2.1.1. Mutations as listed in (a), (b), (e), (g) of **claims 9-13** are shown to result in a higher yield of the enzyme, the mutations as listed in (d), of **claims 9-13** are shown to result in a higher yield and higher activity of the enzyme (see examples 2-5). In this field it is, however, a general aim to obtain high yields and a high activity of recombinantly produced enzymes. The skilled person would, therefore, subject wild type hAGT or the mutants from **D1 or D2** to in vitro mutagenesis methods known in the prior art (see e.g. D3, D4), in order to obtain higher yields of the mutant enzyme that shown high activity. Mutations leading to high yields and high activity of (I) the wild type hAGT or (ii) in combination with the mutations of **D1** (mutations (C) in claim 10) or **D2** (mutations (f) in claims 9 and 10) are, therefore, not inventive.

The specific mutations listed in (a), (b), (d) (e), (g) of claims 9-13 are therefore not inventive.

2.1.2. The mutants of claims 14-30 represent specific embodiments of the non-inventive mutations listed in claims 9-13. Said mutants are therefore also not inventive.

2.1.3. The mutations listed in (d) of claims 9-13 are also described to have decreased activity towards the natural substrate O6-alkyl-N9-deoxyribosylguanine or N9-cyclopentyl-O6-benzylguanine and with oligonucleotides (see p. 7 and p. 22, ln. 4-10). The specification does however, not show any specific further effect or advantage resulting from these characteristics that could confer inventiveness. Said features can therefore not be taken into consideration when examining the claimed subject matter for inventiveness.

Claims 9-30 are therefore not inventive.

2.2. The specification does not show any effect relating to the mutation Pro140/Asn157/Ser159 replaced by Phe/Arg/Glu as described in (f) of claims 9, 10, (see also Tab. 1 in D2 "FREG") . Said mutation is therefore regarded as an arbitrary modification of known subject matter that does not relate to any specific effect. Such an arbitrary modification does, however, not involve an inventive step. The combination of said mutation with any other known or non-inventive mutations as listed in (a)-(e) or (g) of claim 9 (supra), is therefore not inventive. **Claims 9 and 10** are also because of this reason not

inventive.

2.3. The subject matter of **claims 32-36** represent routine modifications that do not involve an inventive step. Said claims are therefore not inventive.

Re Item VIII

Certain observations on the international application

Art. 6 PCT Clarity

Claim 9-13 say that "mutations should be selected from..." It is however not clear if the mutations should be selected from any of the mutations mentioned in said claims or if only one of the mutation should be selected from (A) - (G). The subject matter of said claims is therefore not clearly defined.

The attention of the applicant is drawn to the fact that a reply to this opinion is only expected if he intends to file a chapter II demand.